

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.807.170>

## Evaluation of Pathogenicity of Four Different *Alternaria brassicae* Isolates on Three *Brassica* coenospecies

Prashant Yadav<sup>1</sup>, Zahoor Ahmed Mir<sup>1</sup>, Sajad Ali<sup>2</sup> and Anita Grover<sup>1\*</sup>

<sup>1</sup>ICAR - National Institute for Plant Biotechnology, New Delhi, India

<sup>2</sup>Centre of Research for Development, University of Kashmir, Jammu and Kashmir, India

\*Corresponding author

### ABSTRACT

Indian mustard (*Brassica juncea*) is the second most important edible oilseed crop in the country after soybean. Mustard is an important source of healthy edible oil in the northern states of the country viz., Uttar Pradesh, Rajasthan, Uttarakhand, Haryana, Punjab and Bihar. The production of edible oil in the country is lagging behind the growing demand by ever increasing population and therefore increasing its import. Preventing the loss by various biotic and abiotic stresses is one of the ways to increase the production of mustard oil. Among the various biotic stresses faced by mustard crop in the country, *Alternaria* blight caused by a necrotrophic fungal pathogen *Alternaria brassicae* is an important economic disease. To develop the disease-resistant cultivars, it is important to select the genotypes against most virulent pathogen. For this, we have studied the pathogenicity of four different isolates DEL, PNT, MRT and ND (named according to place of collection) of *A. brassicae* on three different *Brassica* coenospecies viz., *B. juncea*, *Sinapis alba* and *Camelina sativa*, having different degree of resistance against this pathogen. Highest pathogenicity was observed in PNT isolate of *A. brassicae* producing largest size lesion [1.23 cm ( $\pm 0.24$ )] in *B. juncea* whereas, DEL isolate produced smaller size lesion [0.96 cm ( $\pm 0.21$ )] at 12 days post inoculation. Highest number of secondary spots and distal infected leaves were observed with PNT [8.7( $\pm 1.7$ ) and 5.1( $\pm 0.19$ ) respectively]. *C. sativa* never produced any secondary infection or distal infected leaves against any isolate upto 12 dpi. These results concluded that among the four different *A. brassicae* isolates, the PNT isolate was highly pathogenic followed by ND and MRT isolate. The DEL isolate was found least pathogenic in *B. juncea*.

#### Keywords

*Alternaria brassicae*, Disease resistance, Mustard, Pathogenicity

#### Article Info

##### Accepted:

12 June 2019

##### Available Online:

10 July 2019

### Introduction

Among the different edible oilseed crops grown in the country (viz., soybean, rapeseed-mustard, groundnut, sunflower, sesame, linseed, palm oil etc), Indian mustard (*B. juncea*) is the second most important edible oilseed crop after soybean. *B. juncea* is a good

source of healthy fatty acids like omega-3 and omega-6 fatty acids, and is very popular edible oil in northern states of the country. Presently, mustard is grown in area of 6.0 Mha with total production and productivity of 7.9 MMT and 1319 Kg/ha respectively (FAO. 2017). The production of edible oil in the country is lagging behind the consumption rate forcing

India to import 14.5 MMT of edible oil in 2017-18 (The Solvent Extractors Association of India, 2018). Food and Agriculture Organization has estimated that India has highest edible oil consumption growth rate (3.1%) in the world, that will lead the per capita consumption upto 24 Kg/person by 2027 (FAO, 2018). The production of mustard can be enhanced by preventing the losses due to various biotic and abiotic stresses. *Alternaria* blight is a major biotic factor causing yield losses upto 36.8 % in severe conditions (Bal and Kumar 2014).

*Alternaria brassicae* is the causal organism of *Alternaria* blight and it is a member of phylum Ascomycota. It is a necrotrophic fungal pathogen which kills its hosts by producing fungal toxins (Pedras and Smith, 1997). It produces brown-lack typical concentric ring spots popularly known as 'target board symptoms'. *A. brassicae* asexually produces large, multi-cellular and muriform conidia. The biochemical control (fungicides) of *A. brassicae* is available but its application leads to the increased cost of cultivation and environmental concerns.

Therefore, breeding for *Alternaria* resistant cultivars is a best strategy to prevent the losses through *Alternaria* blight. But in Brassica primary gene pool, the *Alternaria* resistant source is absent. However, some member of the Brassica coenospecies (*viz Sinapis alba* and *Camelina sativa*) possess resistance against *A. brassicae* (Sharma *et al.*, 2002). *S. alba* and *C. sativa* are minor crops, currently grown in limited are for seeds and oil in Europe, USA, Canada and Australia. To develop the disease resistant cultivar, it is very important to test the improved genotype against virulent strain/isolates of the pathogen. For this, we studied the virulence of four different isolates of *A. brassicae* on the three Brassica coenospecies from 3 to 12 days post inoculation (dpi).

## Materials and Methods

### Plant growth and infection

Seeds of all the three species (available in the lab) were grown in plastic pots filled with soil and vermicompost at the nethouse of the ICAR - Indian agricultural research Institute, New Delhi during the winter season of 2018-19. *A. brassicae* isolates were collected from four different locations of the North India *viz* ICAR-IARI, New Delhi (isolate named as DEL), Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (PNT), Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (MRT), and Narendra Deva University of Agriculture and Technology, Ayodhya (ND). Mustard leaves infected with *A. brassicae* were collected from the fields of the above locations and pure culture was isolated on radish extract agar media. Spore suspension was prepared from the *A. brassicae* plates and concentration was adjusted to  $5 \times 10^3$  spores/ml. Plants of the three Brassica coenospecies were infected with *A. brassicae* spores under control temperature (25–30 °C) and humidity (100% RH) conditions in a poly-house.

### Disease progression

*A. brassicae* infected plants of *B. juncea*, *S. alba* and *C. sativa* were observed for the disease progression from 3–12 days post inoculation. For studying the virulence of *A. brassicae* on the three Brassica coenospecies, parameters like lesion size, number of secondary spots and number of distal infected leaves were recorded at 0, 3, 6, 9 and 12 days post inoculation. Lesion size (in cm) measured with the help of a 6 inch scale at time interval. The spots appearing alongside the primary inoculation as result of disease progression were considered as secondary spots. For disease scoring, a modified rating scale (0–6)

was used based on the system given by Conn *et al.*, (1990). Necrotic spots along with the surrounding yellowish necrotic area were considered as area under the pathogen attack. The non-inoculated leaves from the same plants were considered as distal leaves and distally infected leaves were considered as a parameter to study the virulence of each isolates of *A. brassicae*.

### Statistical analysis

The experiment was conducted in a completely randomized design (CRD) and three replications were taken for each observation. Five leaves from each species for all the parameters were taken in one replication. Statistical analysis was done by using R software.

### Results and Discussion

Indian mustard is an important source of edible oil in the country and its productivity is hampered by the Alternaria blight disease (Meena *et al.*, 2016). To identify the high pathogenic strain/isolate of *A. brassicae*, four different isolates (PNT, ND, NRT and DEL) were studied on three *Brassica* coenospecies (*B. juncea*, *S. alba* and *C. sativa*). Disease parameters like lesion size, number of secondary spots and number distal infected leaves were studied from 3-12 days post inoculation. Lesion size was found highest in with PNT isolate [1.23 ( $\pm 0.24$ )] whereas lowest lesion size was observed with DEL isolate [0.96 ( $\pm 0.21$ )] in *B. juncea* leaves at 12 dpi. Lesion size in *S. alba* and *C. sativa* was observed highest with PNT infection whereas with DEL isolate, no disease spots were observed in *C. sativa*. Sharma *et al.*, (2002) had observed similar results in case of *S. alba* and *C. sativa* against *A. brassicae*.

Secondary infection appeared as early as 6 dpi in *B. juncea* with PNT [8.7( $\pm 1.7$ )] isolate of *A. brassicae*. In *B. juncea*, lowest number of

secondary spots was observed with MRT [7.0( $\pm 2.2$ )] isolate. No secondary infection was observed in *C. sativa* with any of the isolates indication high resistance of *C. sativa* against *A. brassicae*.

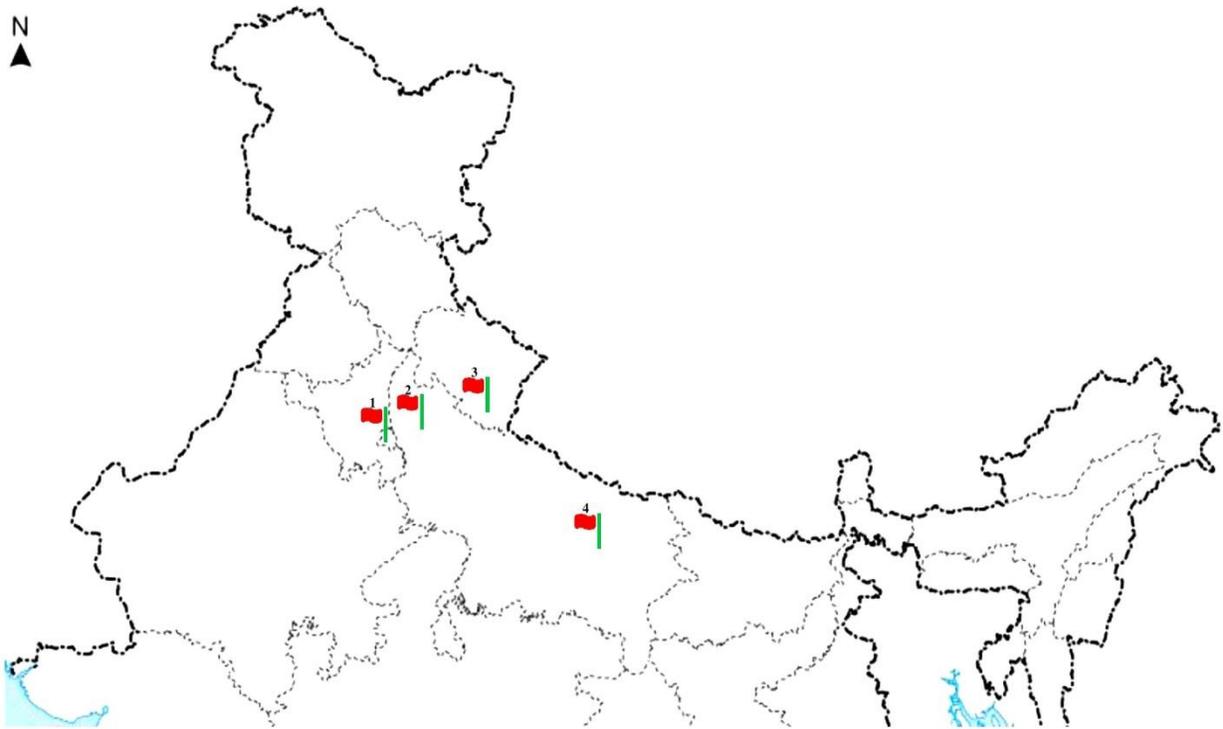
For *S. alba*, highest and lowest number of secondary infection was observed with PNT [2.7( $\pm 0.6$ )] and DEL [1.6( $\pm 0.8$ )] isolates respectively. Mazumder *et al.*, (2013) reported that the resistance in *S. alba* was due to the involvement of abscisic acid (ABA) mediated pathway and susceptibility in *B. juncea* is due to salicylic acid (SA) mediate defense pathway.

This is may be the reason for observing less number of secondary infections in *S. alba* in comparison to *B. juncea*.

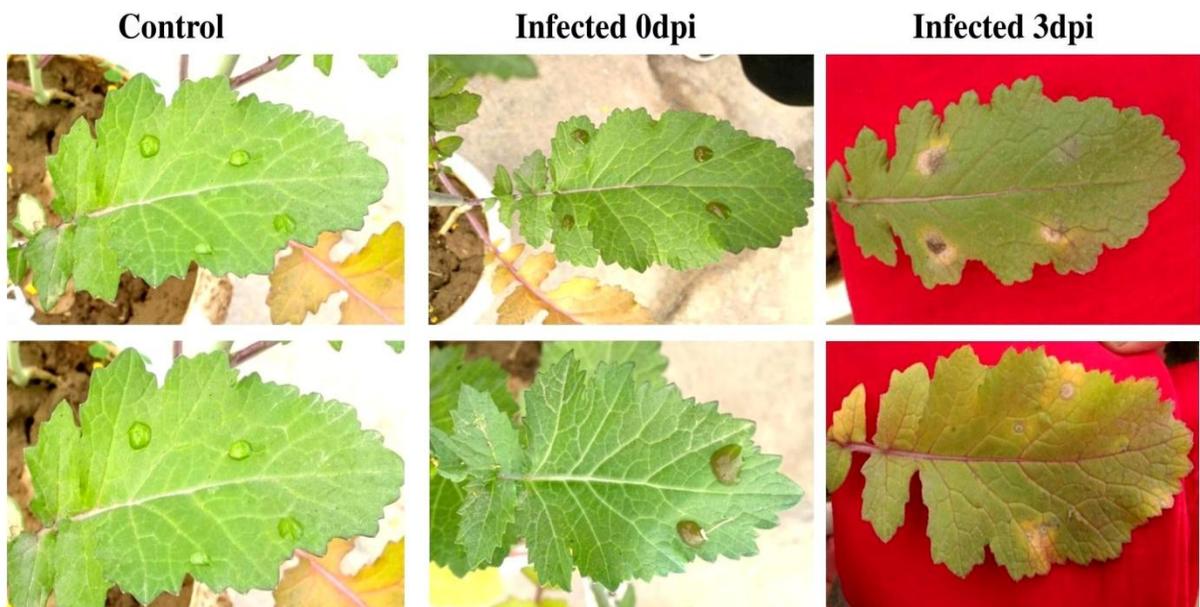
Number of distal infected leaves was observed highest in *B. juncea* with PNT isolate [5.1( $\pm 0.19$ )] and lowest with DEL isolate [3.8( $\pm 0.2.1$ )]. In *S. alba*, highest number of distal infected leaves was reported with PNT [2.3( $\pm 0.94$ )] and lowest with DEL [0.8( $\pm 0.3.2$ )] isolate at 12 dpi. *C. sativa* showed no distal infected leaf by any of the isolate. Based on the scoring of the *A. brassicae* infected leaves of the three *Brassica* coenospecies, *C. sativa* was observed highly resistant (R), *S. alba* moderately resistant (MR) and *B. juncea* highly susceptible (S) against this necrotrophic fungal pathogen.

Sharma *et al.*, (2002) and Nayanakantha (2014) reported similar degree of resistance in these three Brassica coenospecies. *Camelina sativa* produces a phytoalexin called camalexin ((3-thiazol-20-yl-indole) which is known to detoxify fungal toxins (Purnamasari *et al.*, 2015). Meena *et al.*, (2017) studied the virulence of 30 different isolates of *A. brassicae* collected from various geographical locations of the country (Fig. 1 and 2; Table 1).

**Fig.1** Location of the four *A. brassicae* isolates shown on the map. (1=DEL, 2=MRT, 3=PNT and 4=ND)



**Fig.2** Control and *A. brassicae* inoculated leaves of *B. juncea* at 0 dpi and 3 dpi. Water was used in the control whereas *A. brassicae* spore suspension was used for the inoculation



**Table.1** Estimation of pathogenicity of *A. brassicae* isolates (PNT, ND, MRT and DEL) infected leaves of *B. juncea*, *S. alba* and *C. sativa* under control and at 12 dpi. Presented data showed mean± S.E. values estimated by taking 15 leaves from each species (S= susceptible, MR= moderately resistant, and R= resistant)

Species	Mean lesion size (cm)	No of secondary spots	No of infected distal leaves	Rating
<b>Virulence of <i>A. brassicae</i> isolate (PNT)</b>				
<i>B. juncea</i>	1.24 (±0.22)	8.8(±1.7)	5.3(±0.19)	S
<i>S. alba</i>	0.86 (±0.12)	2.8(±0.6)	2.4(±0.94)	MR
<i>C. sativa</i>	0.79 (±0.34)	0.0 (±0.0)	0.0 (±0.00)	R
<b>Virulence of <i>A. brassicae</i> isolate (ND)</b>				
<i>B. juncea</i>	1.20 (±0.22)	8.2(±1.3)	4.8(±0.15)	S
<i>S. alba</i>	0.76 (±0.21)	2.2(±1.6)	2.0(±0.36)	MR
<i>C. sativa</i>	0.38 (±0.16)	0.0 (±0.0)	0.0 (±0.00)	R
<b>Virulence of <i>A. brassicae</i> isolate (MRT)</b>				
<i>B. juncea</i>	1.02 (±0.14)	7.0(±2.2)	4.5(±0.12)	S
<i>S. alba</i>	0.81 (±0.22)	2.1(±1.2)	1.8(±0.24)	MR
<i>C. sativa</i>	0.32 (±0.14)	0.0 (±0.0)	0.0 (±0.00)	R
<b>Virulence of <i>A. brassicae</i> isolate (DEL)</b>				
<i>B. juncea</i>	0.96 (±0.21)	7.2(±1.4)	3.8(±0.2.1)	S
<i>S. alba</i>	0.62 (±0.13)	1.6(±0.8)	0.8(±0.3.2)	MR
<i>C. sativa</i>	0.0 (±0.00)	0.0 (±0.0)	0.0 (±0.00)	R

They observed that the isolates from the Pantnagar were grouped into virulent category whereas, isolated from the Assam, Meghalaya, Nagaland, Bharatpur and Kangra were grouped into highest virulent category. Our study is on isolates from three Northern states of India in contrast to Meena and coworker's work on isolates from different Indian states including North-East Indian states. Based on these parameters, isolate PNT was found highly virulent in *B. juncea* (as also reported by Meena *et al.*, 2017) followed by ND and MRT isolates. DEL isolate was found least virulent in *B. juncea* among the four different isolates. Pramila *et al.*, (2014) also found that the pathogenicity of *A. brassicae* isolate from Pantnagar was higher among different isolates studied from the Uttarakhand. Present study shows that there is variability in the virulence of different isolates of *A. brassicae* on the three *Brassica*

coenospecies. Our research conclude that the highest pathogenic strain/isolate of *A. brassicae* was from the Pantnagar, Uttarakhand and it can be used for the future screening methods to develop *A. brassicae* resistant lines of Indian mustard.

### Acknowledgment

Authors are thankful to the Project Director, National Institute for Plant Biotechnology, New Delhi, for providing research facilities and equipments.

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#### How to cite this article:

Prashant Yadav, Zahoor Ahmed Mir, Sajad Ali and Anita Grover. 2019. Evaluation of Pathogenicity of Four Different *Alternaria brassicae* Isolates on Three *Brassica* coenospecies. *Int.J.Curr.Microbiol.App.Sci*. 8(07): 1426-1431. doi: <https://doi.org/10.20546/ijcmas.2019.807.170>